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Classification of the Organisms Important in Dairy Products

II. Pseudomonas fragi

By R. V. HUSSONG, H. F. LONG AND B. W. HAMMER

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

R. E. BUCHANAN, Director

DAIRY INDUSTRY SECTION



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CONTENTS

	Page
Introduction	119
Experimental	119
Distribution of <i>Pseudomonas fragi</i>	119
<i>Ps. fragi</i> in normal dairy products	119
<i>Ps. fragi</i> in abnormal dairy products	120
General description of <i>Pseudomonas fragi</i>	122
S (smooth) type	123
O (intermediate) type	124
R (rough) type	124
Identity of the organism	125
Isolation of <i>Ps. fragi</i>	125
Variation in the types of colonies produced by <i>Ps. fragi</i>	126
Colony variation in cultures purified by plating	126
Colony variation in cultures purified by single cell isolation	128
Differences in biochemical activities of variants of <i>Ps. fragi</i>	129
Ability of <i>Ps. fragi</i> and the various colony types obtained from it to hydrolyze fat	129
Action of <i>Ps. fragi</i> and the various colony types on the nitrogenous constituents of milk	130
Action of <i>Ps. fragi</i> in butter made from pasteurized cream to which the organism had been added	132
Changes in numbers of <i>Ps. fragi</i> in butter	132
Changes in the total and volatile acidities of butter containing <i>Ps. fragi</i>	133
Changes in flavor of butter containing <i>Ps. fragi</i>	135
Summary	136
Literature cited	136

Classification of the Organisms Important in Dairy Products¹

II. *Pseudomonas fragi*

BY R. V. HUSSONG, H. F. LONG AND B. W. HAMMER

In the studies at the Iowa Agricultural Experiment Station on the bacteria in normal and abnormal dairy products, an organism that produces a rather characteristic change has been encountered over a period of years. On comparatively young plates and in litmus milk it commonly develops a sweet, ester-like odor resembling that of the flower of the common May apple (*Podophyllum peltatum*). Cultures have been isolated in large numbers from various defective dairy products, especially those held at low temperature, and it appears that the organism is of considerable practical importance. A study of the morphology, cultural characters, biochemical features and growth conditions of many cultures indicates that, although the reactions are not always identical, the cultures constitute but a single species. The variations involve especially colony appearance, action on litmus milk and action on fat. The species was identified as *Pseudomonas fragi*; it has been investigated from the standpoint of distribution, identification and action on various dairy products, and the results are presented herein.

EXPERIMENTAL

DISTRIBUTION OF *PSEUDOMONAS FRAGI*

During the plating of numerous samples of milk, cream, butter and other materials, many cultures of *Ps. fragi* have been encountered. The products examined were from various points in Iowa and from certain other states.

PS. FRAGI IN NORMAL DAIRY PRODUCTS

Ps. fragi was occasionally found among the cultures obtained when colonies were picked from plates poured for determinations of the numbers of bacteria in such products as milk, cream and butter. The plates were incubated at 21° C. for 4 to

¹ Projects 113 and 119 of the Iowa Agricultural Experiment Station.

5 days. Since *Ps. fragi* does not grow at 37° C. plates poured for standard counts on milk and cream do not yield it.

In a study of the numbers of bacteria in samples of ice cream, one set of plates was incubated at about 7° C. Colonies suggesting *Ps. fragi* appeared on these plates and when inoculated into litmus milk the expected changes occurred. The organism was obtained from 12 samples of ice cream, 11 being from Iowa and 1 from Tennessee.

PS. FRAGI IN ABNORMAL DAIRY PRODUCTS

MILK

Various samples of abnormal milk were examined for *Ps. fragi*. The samples can be divided into those which were abnormal when received and those held at relatively low temperatures until spoilage occurred.

Two samples of ropy milk were examined. One of these was from a plant in another state which was encountering ropiness in pasteurized milk. Although *Alcaligenes viscosus* was responsible for the ropy condition, cultures of *Ps. fragi* were also isolated. The second sample of ropy milk was from an Iowa city. Many extremely ropy colonies developed on plates poured with this sample. The colonies were identified as *Ps. fragi*, and this organism was considered to be the cause of the ropiness.

Since *Ps. fragi* is psychrophilic and rather regularly present in milk, raw samples held at low temperatures frequently developed a May apple odor and later became rancid. In many instances the organism was isolated from samples of pasteurized milk that had been held at low temperatures. Some of these possessed the May apple odor while others were rancid. *Ps. fragi* is killed by relatively low pasteurization exposures, so its presence in pasteurized materials indicates contamination after heating. Occasionally, samples of skim milk held in a refrigerator showed defects suggesting *Ps. fragi*, and on plating such material cultures of the organism were obtained.

CREAM

Many samples of pasteurized cream were received that had undergone a change in flavor while being held in refrigerators in homes. Usually the cream possessed a typical May apple odor which was followed by rancidity. This defect suggested *Ps. fragi*, and the organism was generally found in large numbers.

COTTAGE CHEESE

On several occasions the cottage cheese manufactured in an Iowa plant developed an odor resembling that produced by *Ps. fragi*. Upon plating such samples the organism was usually isolated.

BULK CONDENSED MILK

Milk evaporated to different densities and held in 10-gallon cans at a temperature slightly above 0° C. developed the May apple odor after several days; the odor was especially pronounced in the milk with the lowest solids content. *Ps. fragi* was isolated from milk with both high and low concentrations of solids.

BUTTER

Seventeen samples of commercial butter in a series sent to the laboratory for various types of examinations were more or less rancid when received; 5 of these were from large creameries, 11 were from small creameries and the source of 1 was unknown. *Ps. fragi* was isolated from 4 of the 5 samples from large creameries; these came from Illinois, Kansas, Minnesota and Virginia. Each of the 11 samples from small creameries yielded the organism; all of these were from Iowa.

In connection with studies on the spoilage of unsalted or low salted butter from an Iowa creamery it was noted that the defect suggested *Ps. fragi*. Some of these samples were rancid, while others suggested a May apple odor. On plating the butter *Ps. fragi* was almost always found in large numbers.

Butter sent to the Iowa Butter Control Laboratory from various plants in the state for analysis is shipped without refrigeration and, occasionally, a sample shows rancidity when received. *Ps. fragi* has been isolated from many of these defective samples over a period of years.

In a study on keeping quality, butter from certain Iowa creameries was held at 21° C. and examined after various periods. Defects suggesting *Ps. fragi* developed in some of these samples, and cultures of the organism were obtained on plating.

Butter is frequently submitted to the Dairy Industry Section with the request that the cause of the off flavor be determined. *Ps. fragi* is often found responsible. Most of the samples from which the organism is isolated possess a May apple odor or are rancid; occasionally, it is obtained from cheesy butter, although the relationship to the cheesiness is not always clear. The butter containing the organism is usually unsalted or has a relatively low salt content. The variation in the action of *Ps. fragi* on butter is in agreement with variations noted in colony appearance and in the action on litmus milk and fat. Two samples received from butter marketing organizations illustrate the importance of *Ps. fragi* in butter.

One sample was rancid. The total yeast and mold count was 17 per ml. On beef infusion agar containing skimmilk and dispersed fat (6), the total bacterial count was 22,900,000 per ml., the proteolytic count 3,800,000, and the lipolytic count 3,600,000.

The appearance of most of the colonies on the plates and the odor of the plates suggested *Ps. fragi*; when representative colonies were picked into litmus milk the reactions obtained were those of *Ps. fragi*. Experimental churnings were made with two lots of cream, each inoculated with a culture of *Ps. fragi* isolated from the plates. The butter containing one culture became rancid in 1 to 2 days at 21° C., while that containing the other culture developed a May apple odor, and rancidity did not develop on extended holding. The colonies on plates poured with the rancid sample were actively lipolytic while those on the plates poured with the other sample showed little or no lipolysis.

Another sample was slightly cheesy. The total bacterial count of the butter on beef infusion agar plus skimmilk at 21° C. was 15,100,000 per ml., and the proteolytic count was 200,000; since no rancidity was noted, a lipolytic count was not made. The plates had a varied flora, and the odor did not suggest *Ps. fragi*; however, when colonies were picked into litmus milk a number of cultures of this organism were obtained. Experimental butter made with three lots of cream, each inoculated with a *Ps. fragi* culture from the cheesy butter, showed various defects; one sample became rancid, another cheesy to rancid and the third cheesy. Because of the number of species present in the original sample of butter the organism causing the defect could not be definitely established, but probably *Ps. fragi* was involved.

MISCELLANEOUS MATERIALS

Cultures of *Ps. fragi* were also isolated from such sources as homogenizer packing, water from a lake on the Iowa State College campus, and sheep fat that had been held in the laboratory for several weeks.

The data indicate that *Ps. fragi* is a widely distributed species that, in many instances, is responsible for defects in dairy products, especially those held at relatively low temperatures for extended periods. It has been frequently obtained from defective milk, cream, cottage cheese, bulk condensed milk and butter. Hard cheeses have not been studied for the presence of this organism, but Hansen (4) found that when it was inoculated into pasteurized milk used for cheddar cheese the quality of the cheese was impaired. The nitrogenous decomposition of the cheese containing *Ps. fragi* was not greatly affected as compared with the control.

GENERAL DESCRIPTION OF *PSEUDOMONAS FRAGI*

The organism produced three distinct types of colonies—S (smooth), O (intermediate) and R (rough)—when grown on the usual agar media. Since the types differ in biochemical ac-

tivity, especially in the action on litmus milk and on fat, separate descriptions are given. In describing the O and R types, only the characters in which they vary from the S type are presented.

S (SMOOTH) TYPE

MORPHOLOGY (CULTURES GROWN AT 21° C.)

Form and size: Rods; 0.5 to 1.0 by 0.75 to 4.0 microns when grown 1 day on beef infusion agar.

Arrangement: Singly, pairs, and chains of varying length. Extremely long chains often noted in 1-day cultures on beef infusion agar.

Motility: Motile; commonly a polar flagellum.

Staining reactions: Stains readily with common stains; gram negative.

Spores: None observed.

CULTURAL CHARACTERISTICS (CULTURES GROWN AT 21° C.)

Agar slant: After 1 to 2 days on beef infusion agar an abundant, spreading, raised, white and shiny growth. Most cultures butyrous, others viscid. Generally possessed a sweet, ester-like odor resembling that of the flower of the May apple.

Agar stab: Surface growth on beef infusion agar with a smaller amount of growth extending along the line of inoculation.

Agar colony: On beef infusion agar a convex, glistening, smooth-surfaced and opaque colony; most cultures butyrous, others viscid.

Gelatin stab: Crateriform to stratiform liquefaction in 3 to 4 days.

Beef extract broth: Turbidity and sediment with a thin pellicle.

Potato: Echinulate to arborescent, raised, glistening, white growth, becoming slightly brown on extended incubation.

Litmus milk: First change generally the formation of an acid ring at the surface and later an acid coagulum at the surface next to the wall of the tube. After approximately 10 days, acid noted in the lower portion of the tube. After 2 to 3 weeks coagulation generally complete throughout the milk with some digestion usually evident. Ropiness rarely produced. A characteristic May apple odor given off.

BIOCHEMICAL FEATURES (CULTURES GROWN AT 21° C.)

Indol: Not produced.

Nitrates: Not reduced.

Hydrogen sulfide: Not produced in agar.

Methyl red reaction: Negative.

Voges Proskauer reaction: Negative.

Ammonia: Produced from peptone.

Fermenting power: Dextrose and galactose fermented with the production of acid but no gas. Arabinose attacked with the production of acid but no gas by some strains but not by others. Neither acid nor gas produced from glycerol, inulin, lactose, levulose, maltose, mannitol, raffinose, salicin and sucrose.

Hydrolysis of fat: Fat hydrolyzed.

GROWTH CONDITIONS

Oxygen relationships: Aerobic.

Growth temperatures: Growth at 10° C., 30° C., and temperatures in between. No growth at 37° C.

Heat resistance: Very sensitive to heat. Of 5 cultures carried on artificial media for a considerable period, 1 survived 62.2° C. for 10 minutes. Of 12 freshly isolated cultures 9 did not survive 61.1° C. for 3 minutes while 1 survived 3 but not 5 minutes, 1 survived 5 but not 10 minutes, and 1 survived 10 but not 20 minutes. Organism killed by incubation of slope cultures for several days at 37° C.

O (INTERMEDIATE) TYPE

The O type of *Ps. fragi* is characterized as follows:

Agar colony: A thin, translucent, smooth-edged, smooth-surfaced and butyrous growth on beef infusion agar.

Litmus milk: Rarely coagulated but generally slightly reduced in the bottom of the tube.

Hydrolysis of fat: Fat not hydrolyzed by most strains.

R (ROUGH) TYPE

Three types of R colonies, varying in degrees of roughness and opacity, were found. They are characterized as follows:

Agar colony: R1 colony was thin, translucent, spreading, flat and bluish with irregular edge and a smooth surface on beef infusion agar. Possibly an intermediate between the S and R colonies.

R2 colony was thin, translucent, rough-edged, wrinkled, and larger than S type; generally very tough in consistency. Tinged with brown on extended incubation, and having the appearance of a thin, transparent, brown membrane.

R3 colony was opaque, white, raised, rough-surfaced, with an irregular margin; encountered only a few times.

Litmus milk: Action of R colonies on litmus milk differed only in degree from that of S colonies. Less proteolytic than the S type and more proteolytic than the O type.

Hydrolysis of fat: Fat hydrolyzed by some R types but not by others.

IDENTITY OF THE ORGANISM

The description of the organism agrees rather closely with organisms described by Eichholz and Grüber as producing a strawberry-like odor. Since an odor is rather difficult to describe, too much importance should not be attached to it, and more emphasis should be placed on biochemical activity.

Eichholz (1) isolated an organism, which he designated *Bacterium fragi*, from milk held several days at 3.5° to 7.0° C. It produced a strawberry aroma, did not develop spores, and formed rosette and daisy-like patterns on gelatin. On agar streaks the growth was easily removed and was neither sticky nor slimy. No fluorescence was observed. Neither acid nor gas was formed in milk, and the milk became alkaline on extended holding. The organism was killed by heating for 30 minutes at 50° C. or 10 minutes at 75° C.

Grüber (2) isolated an organism producing a strawberry odor and named it *Pseudomonas fragariae*. It came from beets and was a non-spore forming, fluorescent, non-gelatin liquefying species, with 1 to 9 polar flagella. The colonies were described as dewdrop like, bluish, round, raised, shining and translucent. Both salted and unsalted butter containing the organism developed a strawberry odor. Later Grüber (3) isolated an organism, *Pseudomonas fragariae* II, from pasteurized milk held for a considerable period which had developed a strawberry odor. The odor was attributed to growth of the organism. On gelatin plates the colonies were large, round, arched, dirty-white and glistening. The organism was rod-shaped, motile by means of a polar flagellum, liquefied gelatin and coagulated milk by the production of acid. The optimum growth temperature was 18° to 22° C., and there was no growth above 34° C.

Hussong (5) considered that the three species were either identical or closely related and that each could be identified as one of the colony types of the organism producing a May apple odor. Thus *Bact. fragi* Eichholz was identified as the R colony type of the May apple organism, *Ps. fragariae* Grüber as the O type, and *Ps. fragariae* II Grüber as the S type. Since the characters of the species indicate that it belongs in the genus *Pseudomonas* and the name *Bact. fragi* was first applied by Eichholz, Hussong proposed the combination *Pseudomonas fragi* (Eichholz) Hussong.

ISOLATION OF *PS. FRAGI*

At the Iowa Agricultural Experiment Station *Ps. fragi* is easily obtained if a dairy product having a May apple odor is plated and promising colonies picked. In some cases rancid products yield the organism, and, occasionally, it is present in

cheesy butter. It is also readily obtained by placing samples of normal, raw milk or cream at approximately 5° C. until a May apple odor or rancidity develops and then plating. Since the organism is definitely psychrophilic such an enrichment procedure aids materially in its isolation. Various agars can be used for plating, but beef infusion agar is especially useful. The plates are incubated at approximately 21° C. instead of 37° C.

VARIATION IN THE TYPES OF COLONIES PRODUCED BY *PS. FRAGI*

Four cultures of *Ps. fragi* were selected from those on which detailed observations had been made and used for a study of colony variation. Each of the cultures hydrolyzed fat and produced rancidity in unsalted butter made from cream inoculated with it. The sources of the cultures were as follows:

Culture A was isolated from rancid butter and was considered to be the cause of the rancidity.

Cultures B and C were isolated directly from plates poured with two samples of normal ice cream.

Culture D was isolated from ropy milk and was considered the cause of the abnormality.

COLONY VARIATION IN CULTURES PURIFIED BY PLATING

Variation in the types of colonies was studied on beef infusion agar (pH 6.8) plates poured with 2-day cultures of each organism and incubated at 21° C. After a few platings the amount of inoculum could be so controlled that the colony distribution was satisfactory. When colonies had developed on the plates a system of selective picking was used whereby one of each of the colony types appearing on the plates was picked into litmus milk. This procedure is referred to as "successive platings" in the following consideration. These new litmus milk cultures formed the inocula for the next plates in the series. In no case was any material such as dye, inorganic salt, or disinfectant employed to bring about changes in colony form although litmus was used in the milk.

CULTURE A

After incubating 2 days, plates poured with culture A showed a large percentage of S colonies although a few O and R1 colonies were present. A representative of each of these types was picked into litmus milk and incubated at 21° C. for 2 days. These cultures served as material for the platings which are recorded below.

S TYPE

An S colony from the original plating was passed through eight successive platings. The colonies appearing on the plates of the entire series were largely of the same type as the parent

colony. On five of the eight sets only S colonies were observed. Variant colonies appeared on platings 2, 3 and 7. Those appearing on platings 2 and 3 consisted of a few O colonies which were picked into litmus milk, passed through several successive platings, and the plates examined for variant colonies; none of them showed any variation in colony type. The variant colonies appearing on plating 7 consisted of a few O and a few R3 colonies. These were not studied further.

An S culture from the eighth plating was used for single cell isolation.

O TYPE

An O colony from the original plating was passed through eight successive platings. There was no evidence of colony variation on any of the plates in the series. A colony from the last plating was used for the isolation of single cells.

R1 TYPE

An R1 colony from the original plating was passed through eight successive platings and no variations were noted. A culture from the last plating was used for the isolation of single cells.

CULTURE B

Culture B was repeatedly plated to insure its purity before studying colony variation. Here, as with culture A, there were three types of colonies, S, O and R1. Each of these types was carried through eight successive platings and the plates examined for evidence of colony variation. None was observed in any of the plates in the series. A culture from the eighth plating of the S type was used for the isolation of single cells.

CULTURE C

After purification, the first plating of culture C yielded two types of colonies, S and O. Both of these types were carried through a series of eight platings. There was no evidence of variation on any of the plates in the series. A colony from the eighth plating of each type was used for the isolation of single cells.

CULTURE D

Following purification, the first plating of culture D yielded three colony types, S, O and R1. Each of these colony types was used for subsequent platings.

S TYPE

The S colony was carried through eight successive platings. The first plating in the series was the only one which gave any evidence of variation. The variants consisted of a few O colonies which, when plated, yielded only O colonies.

O TYPE

No variation was observed on any of the plates in the series of eight platings to which the O colony was subjected.

R1 TYPE

The R1 type was passed through eight successive platings, and the only variation noted occurred on the first plating in the series. The variants consisted of a few S colonies which did not show variation during a series of platings.

The data on the cultures purified by repeated plating show that there was considerable variation in the colonies obtained and suggest that the cultures may not have been pure.

COLONY VARIATION IN CULTURES PURIFIED BY SINGLE CELL ISOLATION

The value of purifying bacterial cultures by a technic utilizing single cell isolation has been emphasized by various investigators. Certain of the variant strains of *Ps. fragi* were purified by the method of Wright and Nakajima (8) in which the single cell is inoculated into a small droplet of medium and incubated in a moist chamber. A Leitz micromanipulator was used for carrying out the mechanical part of the process. The variants used for isolation were from the studies on colony variation in cultures purified by plating. Some of the variant cultures produced a slimy material which made single cell isolations impossible since the cells adhered and a droplet containing only one organism could not be obtained.

CULTURE A

With culture A single cell isolations were obtained with S, O and R1 types. On repeated plating the S type yielded S, O, R1, R2 and R3 colonies, the O type yielded R1 in addition to O colonies, while the R1 type yielded R2 in addition to R1 colonies.

CULTURE B

Only the S type was purified by single cell isolation. On repeated plating it did not yield colony variants. This agrees with the results on the S variant of culture B when the plating technic was used for studying variation.

CULTURE C

With culture C single cell isolations were obtained with the S and O types. On repeated plating the S type yielded O in addition to S colonies, and the O type yielded R1 and R2 in addition to O colonies.

In general, the variation in cultures purified by the single cell technic agrees with that in the cultures purified by plating, al-

though the variation was somewhat greater with the former procedure. With some of the strains variation appeared to be more pronounced after extended holding of cultures, either at 21° C. or in a refrigerator, than when transfers were made frequently.

DIFFERENCES IN BIOCHEMICAL ACTIVITIES OF VARIANTS OF *PS. FRAGI*

Many investigators have noted differences in the biochemical activities of the dissociated strains of an organism. Because *Ps. fragi* varied in its ability to hydrolyze fat and in its action on milk, more detailed studies of the effects of the variants on these materials were carried out.

ABILITY OF *PS. FRAGI* AND THE VARIOUS COLONY TYPES OBTAINED FROM IT TO HYDROLYZE FAT

Fat hydrolysis was investigated with the four cultures and their variants that were used in the study on colony variation. Lipolysis was detected by means of Nile blue sulfate (6). The data are presented in table 1.

In general, the S, O, R1, R2 and R3 types isolated from culture A hydrolyzed fat. One strain of the O type was obtained which did not have this ability; it was found on a plate poured with an S culture which hydrolyzed fat, and, morphologically, this O culture did not differ from the O cultures which did hydrolyze fat. The S and R1 colonies from culture B hydrolyzed fat while the O colony did not. Both S and R1 colonies from culture C hydrolyzed fat while the O colony did not; later, an R2 colony was found which was not lipolytic. The S colony of culture D was lipolytic, while the O and R1 types were not.

The data indicate that there is a distinct difference in the lipolytic ability of the variants of *Ps. fragi*. In addition there may be a difference in the lipolytic activity of the same colony types obtained from different cultures.

TABLE 1. ABILITY OF THE FOUR CULTURES AND THEIR COLONY VARIANTS*
TO HYDROLYZE FAT.

+ = fat hydrolyzed;
- = fat not hydrolyzed

Culture	Action of original culture	Action of colony variants				
		S	O	R1	R2	R3
A	+	+	+ and -†	+	+	+
B	+	+	-	+		
C	+	+	-	+	-	
D	+	+	-	-		

*The cultures and their variants were those used in studying colony variation.

†Only one non-lipolytic strain isolated.

ACTION OF *PS. FRAGI* AND THE VARIOUS COLONY TYPES ON
THE NITROGENOUS CONSTITUENTS OF MILK

The protein breakdown in skimmilk was investigated with the four cultures used in studying colony variations and with variants obtained from them. After incubation of the milk cultures for 7 days at 21° C. the water lost by evaporation was restored, the cultures were then acidified with glacial acetic acid at the rate of 1 ml. to 250 ml. of milk and heated to 60° C. to flocculate the insoluble material. The cultures were cooled, filtered through paper and the whey analyzed for soluble nitrogen by the Kjeldahl method and for amino nitrogen by the Van Slyke procedure (7). Commonly, soluble nitrogen determinations were made on the original cultures and on variants from platings 1 and 2, while amino nitrogen determinations were made on the original cultures and the variants from platings 1, 2, 3, 4, 6 and 7. The results are expressed as the increase or decrease in the milligrams of nitrogen per 10 ml. of whey over the uninoculated control. The data are given in table 2.

The original cultures showed considerable variation in the proteolyzing action on milk. Cultures A, B and D gave large increases in soluble and amino nitrogen while culture C gave relatively small ones.

The data on culture A indicate that there was a marked difference in the proteolytic activity of the various colony types. The increases in soluble nitrogen produced by the S and the R colonies are comparable to those brought about by the original culture, while the O colony produced a much smaller increase. The S colony obtained from the second plating gave the largest increase in soluble nitrogen of any of the colony types examined in the various trials, while the two soluble nitrogen determinations made on O colonies showed them to be about half as proteolytic as the R type. The amino nitrogen increases brought about by the variant cultures show approximately the same differences as the soluble nitrogen increases. In the six determinations the S colonies caused greater increases than the original culture, the R colonies gave the next largest increases, while the O colonies in all instances brought about the smallest increases.

Culture B did not give values in agreement with those on culture A since there was a very low amino nitrogen increase. However, the various colony types exhibited approximately the same relationships as those of culture A. Large increases in soluble nitrogen were brought about by the S type and fairly large increases by the R type, while the amounts of soluble nitrogen formed by the O type were very low as compared to the original culture and to the O variants of culture A; it should be noted that the O type of culture A was sometimes lipolytic while the O

TABLE 2. ACTION OF THE FOUR CULTURES AND THEIR COLONY VARIANTS* ON THE NITROGENOUS CONSTITUENTS OF MILK.

Culture	Increase over control per 10 ml. of whey							
	Culture A		Culture B		Culture C		Culture D	
	Mg. soluble nitrogen	Mg. amino nitrogen	Mg. soluble nitrogen	Mg. amino nitrogen	Mg. soluble nitrogen	Mg. amino nitrogen	Mg. soluble nitrogen	Mg. amino nitrogen
Original	16.16	1.88	21.10	0.76	1.76	0.17	12.63	1.49
Plating 1	S	3.80	15.18	2.06	11.05	1.03	6.09	0.17
	O	1.01	0.36	-0.11	0.05	0.03	-0.56	-0.15
	R†	2.85	6.55	0.61			0.13	-0.12
Plating 2	S	20.41	3.33	15.82	2.33	13.60	1.43	6.51
	O	8.48	0.84	0.16	-0.10	1.09	-0.08	0.06
	R	17.24	2.98	7.67	0.49		0.18	-0.09
Plating 3	S		3.51	1.97		1.57		0.90
	O		0.80	0.01		0.17		-0.15
	R		2.51	0.63				-0.27
Plating 4	S		3.73	2.38		2.07		1.01
	O		1.01	-0.10		-0.34		-0.04
	R		3.02	0.77				0.04
Plating 6	S		3.44	2.41		0.96		0.65
	O		0.95	-0.06		-0.11		-0.13
	R		2.36	0.47				-0.16
Plating 7	S		2.59	1.79				2.72
	O		0.66	-0.16				-0.31
	R		2.23	0.05				2.97

*The cultures and their variants were those used in studying colony variation.

†The R1, R2 and R3 types are all referred to as R to simplify the table.

type of culture B was not. The S type again produced the highest amino nitrogen increases, while the R type produced relatively low ones. Five of six determinations on O variants showed slight decreases and the remaining determination a very small increase. The slight decreases are probably not of significance, although they were fairly consistent.

The original strain of culture C gave a very slight increase in both soluble and amino nitrogen as compared to cultures A and B, and there is the possibility that it was an O instead of an S type. The S variants from culture C gave much higher soluble nitrogen increases than the original; however, these values were lower than those for the corresponding type of cultures A and B. R colonies from culture C were not available when the determinations were made. The O type again showed small increases in soluble nitrogen. While, in general, the amino nitrogen increases for the variants were low, the increases for the S type were higher than those for the original culture. Three O variants gave slight decreases and two gave slight increases. These changes are so small that they are of little importance but they are of significance when compared to the action of the S cultures.

Culture D gave rather large increases in soluble and amino

nitrogen. In general, the variants were less active than those of the other three cultures. The values for soluble nitrogen show that the S colony was less active than the parent culture, although there was still considerable soluble nitrogen formed. The R and O colonies produced only a very slight change in the amount of soluble nitrogen. Five of the S variants gave considerably lower amino nitrogen increases than the parent culture, while the sixth gave a higher increase. In four of the six determinations on the R type there was a decrease; one of the two remaining determinations showed a very slight increase and the other a relatively large increase. In each of the six determinations on the O variants there was a slight decrease in the amount of amino nitrogen.

The comparative action of the variant types on fat and on the nitrogenous constituents of milk is of interest. The S type regularly possessed a strong lipolytic and proteolytic action. The R type was usually lipolytic and usually actively proteolytic. The O type was generally not lipolytic and was usually weakly proteolytic, although with culture A it was commonly lipolytic and rather actively proteolytic.

ACTION OF *PS. FRAGI* IN BUTTER MADE FROM PASTEURIZED CREAM TO WHICH THE ORGANISM HAD BEEN ADDED

A number of experimental lots of butter were made from pasteurized cream inoculated with *Ps. fragi*, and the butter was studied from the standpoint of the changes occurring in it. Some of the butter was salted and some was unsalted; most of it was made without butter culture, but with two lots 10 percent culture was added to the cream just before churning. The butter was held at about 7° C.

CHANGES IN NUMBERS OF *PS. FRAGI* IN BUTTER

Various lots of the butter were examined for the numbers of bacteria when fresh and again after holding at about 7° C. for different periods. Numbers were determined by the plate count, using beef infusion agar (pH 6.8) and incubating the plates 4 days at 21° C. In the instances in which butter culture organisms were present in the butter along with *Ps. fragi*, only the *Ps. fragi* colonies were counted. The results obtained are presented in table 3.

The counts on the freshly churned butter varied widely. In unsalted butter all of the cultures of *Ps. fragi* showed rapid increases in the numbers of organisms. The increases were very evident after 3 or 4 days, when the first counts following holding were made; after 7 or 9 days the counts were sometimes higher and sometimes lower than those after 3 or 4 days. Counts of over 50 million per ml. were rather common, and a few counts

TABLE 3. NUMBERS OF *PS. FRAGI* IN BUTTER AT VARIOUS AGES.
Butter held at 7°C.

Series	Culture	Salt or butter culture added	Bacteria per ml. of butter		
			Freshly churned	After 3 days	After 7 days
2	1		745,000	52,000,000	100,000,000
	3		940,000	52,000,000	103,500,000
	4		1,080,000	18,000,000	24,000,000
3	2		340,000	18,650,000	
	2	2.5% salt	22,500	140,000	
4	4		295,000	32,500,000	12,750,000
	4	1.0% salt	65,000	4,950,000	800,000
	4	2.5% salt	25,000	385,000	210,000
	4	10.0% butter culture	475,000	42,000,000	11,400,000
			Freshly churned	After 4 days	After 9 days
5*	S		1,425,000	97,500,000	69,000,000
	O		385,000	51,000,000	136,000,000
	R1		1,020,000	35,000,000	22,000,000
	R2		205,000	25,000,000	15,750,000
	R2		11,550,000	51,000,000	23,000,000

*The colony variants were obtained from one original culture.

over 100 million were obtained. In one series of churnings certain colony variants, obtained from one original culture, were used for the inoculation of the cream, and all of them increased rapidly in the butter.

The addition of salt to the butter definitely inhibited the growth of *Ps. fragi*. The inhibition was striking in the one lot of butter involving the addition of 1 percent salt and was still greater in the two lots to which 2.5 percent salt had been added. Counts obtained on the butter with which butter culture was used were as high as those on the control.

CHANGES IN THE TOTAL AND VOLATILE ACIDITIES OF BUTTER CONTAINING *PS. FRAGI*

The changes in the total and volatile acidities of butter containing *Ps. fragi* were determined with a number of the lots. Total acid was determined by titrating 10 gm. of butter in 15 ml. of ethyl alcohol and 35 ml. of ether with N/10 sodium hydroxide; phenolphthalein was used as the indicator. The amount of alkali required is referred to as the acid value of the butter. Volatile acid was determined by steam distilling 500 gm. of butter and titrating the first liter of distillate with N/10 sodium hydroxide, using phenolphthalein. The amount of alkali required is referred to as the volatile acid value.

Table 4 presents the total acid values obtained on two series of churnings. Series 1 involved three cultures of *Ps. fragi*, while series 5 involved several colony variants all of which were iso-

TABLE 4. TOTAL ACID VALUES OF BUTTER CONTAINING *PS. FRAGI*.

Series	Culture used	Ml. N/10 NaOH required per 10 gm. butter after			
		21 days	28 days	42 days	91 days
1	None	1.75		2.1	2.4
	1	10.75		14.4	29.8
	3	18.2		21.4	39.15
	4	16.65		22.5	48.4
5*	None	1.2	1.6		
	S	6.1	9.1		
	O	1.2	1.5		
	R1	6.7	9.1		
	R2	9.0	12.2		
	R2	8.6	12.9		

*The colony variants were obtained from one original culture.

lated from one original culture. The data show that when *Ps. fragi* was added to the cream from which the butter was churned there was a conspicuous increase in the total acid in all cases except when the O type was used (series 5), and in this case the acidity was essentially the same as with the control. Since the O type commonly fails to hydrolyze fat the failure to increase the total acid would be expected. The control butter contained only comparatively small amounts of total acid even after rather long holding.

Data showing the influence of salt on the changes in the total acid values of butter are given in table 5. With each of the four cultures of *Ps. fragi* the total acid values were high after the holding period, whether the butter was unsalted or salted, but in all cases the values were conspicuously higher in the unsalted butter than in the salted. The control butter also showed more total acid after the long holding period (180 days) when it was unsalted than when it was salted.

Table 6 gives the total and volatile acid values obtained in three series of churnings. Series 2 involved four cultures, series 3 and 4 each one culture, either alone or with salt or butter culture. As with the results given in tables 4 and 5, the total acid values were regularly increased when *Ps. fragi* was present in the butter but were less when salt or butter culture was used

TABLE 5. TOTAL ACID VALUES OF UNSALTED AND SALTED BUTTER CONTAINING *PS. FRAGI*.

Series	Culture used	Ml. N/10 NaOH required per 10 gm. butter after 180 days	
		Unsalted	Salted
2	None	4.1	1.6
	1	9.5	6.0
	2	29.1	11.8
	3	15.3	11.5
	4	19.6	12.4

TABLE 6. TOTAL AND VOLATILE ACID VALUES OF BUTTER CONTAINING *PS. FRAGI*.

Series	Culture	Salt or butter culture added	Age of butter in days	Acid values	
				Ml. N/10 NaOH required per 10 gm. butter	Volatile acid*
2	None		40†	2.5	1.0
	1		28	7.8	25.0
	2		14	16.4	81.0
	3		16	8.8	28.0
	4		16	10.0	72.0
3	None		43†	3.4	4.0
	2		36	23.4	129.0
	2	2.5% salt 10 % butter culture	40	5.7	34.0
	2		42	9.7	51.0
	2				
4	None		47†	2.0	3.6
	4		39	17.6	117.0
	4	1.0% salt 10 % butter culture	40	11.7	79.0
	4		42	7.3	39.0
	4				

*Expressed as ml. N/10 NaOH required to neutralize the first liter of distillate when 500 gm. of butter were steam distilled.

†Examined after all the other lots of butter in the series had been examined.

than when it was not. The increase in total acid was always accompanied by an increase in volatile acid, but there was no close correlation between the extent of the two increases. From the total and volatile acid values it is evident that only a very small percentage of the total acid in the butter is volatile under the distillation conditions used. The amounts of total and volatile acid present in the controls were always small compared to the amounts in the butter churned from cream inoculated with *Ps. fragi*.

CHANGES IN FLAVOR OF BUTTER CONTAINING *PS. FRAGI*

Ps. fragi does not always produce the same flavor defect in butter. The common change is the production of a May apple odor followed by rancidity. With a heavy inoculation of the cream and no salt in the butter, experimental churnings often showed the May apple odor in 1 or 2 days at 7° C. and later developed a typical rancidity. The addition of 1 or 2.5 percent salt to the butter delayed the appearance of the off flavor, the delay being greater with the higher salt content than with the lower. When 10 percent butter culture was added to the cream just before churning the rancid flavor developed somewhat less rapidly than when the butter was made without culture.

With certain cultures of *Ps. fragi* the May apple odor or the rancidity may not be evident, and in some instances both are absent and the butter becomes cheesy. In one series of experimental churnings, in which the action of 10 cultures of *Ps. fragi* on unsalted butter was studied, 8 produced cheesiness and 2 ran-

cidity when the butter was held 7 days at 21° C. These results suggest that *Ps. fragi* may cause cheesiness in butter, and, in a few instances, studies on commercial unsalted butter showing cheesiness have indicated that this organism was responsible for the defect. Samples of commercial salted butter showing cheesiness have not contained *Ps. fragi* in sufficient numbers to suggest its relationship to the defect, and in salted butter *Ps. fragi* commonly causes a May apple odor and rancidity rather than cheesiness.

SUMMARY

Ps. fragi is an important organism in dairy products as indicated by its frequent occurrence and the defects produced. It often causes an odor suggesting that of the flower of the May apple and then rancidity; occasionally, the organism is responsible for cheesiness in unsalted butter. Presumably, the same defect is not always produced by *Ps. fragi* because of variations in the species. *Ps. fragi* can frequently be obtained by pouring plates with a product possessing a May apple or rancid odor and picking characteristic colonies. If it is to be isolated from normal milk this should be held at 5° to 7° C. until a defect develops before pouring plates because the psychrophilic character of the organism enables it to outgrow many other species at relatively low temperatures.

The extreme variability of *Ps. fragi*, especially in colony appearance and action on litmus milk and fat, makes the organism difficult to identify until the variations in the colony types are understood.

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